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Genetic Loci Related to Kernel Quality Differences between a Soft and a Hard Wheat Cultivar

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ABSTRACT

Hybridizations between hard and soft wheat types could be a source of novel variation for wheat quality improvement. This study was conducted to identify genomic regions related to differences in milling and baking quality between a soft and a hard cultivar of hexaploid wheat (*Triticum aestivum* L.). A population of 101 double-haploid lines was generated from a cross between Grandin, a hard spring wheat variety, and AC Reed, a soft spring wheat variety. The genetic map included 320 markers in 43 linkage groups and spanned 3555 cM. Quadrumat-milled flour yield, softness equivalent, flour protein content and alkaline water retention capacity were evaluated for three locations and one year, and Allis-Chalmers milling, mixograph, and cookie baking tests were completed without replication. The effect of qualitative variation for kernel texture, caused by the segregation of the *Hardness* gene, was controlled by regression on texture class. The residual variance was used for composite interval mapping, and QTLs on 1A, 1B, 1A/D, 2A, 2B, 2D, 3A/B, 4B, 5B and 6B were detected. The effect of some QTLs was opposite to the direction expected on the basis of parental phenotypes. The hard wheat parent contributed alleles favorable for soft wheat varieties at QTLs on 1AS,L, 1BL-2, and 6B, whereas the soft parent contributed alleles for higher protein content at QTLs on 2BL-1, 4B-1, and 6B and higher flour yield on 2BL-2 and 4B-2. These results indicated that hard × soft wheat crosses have considerable potential for improving milling and baking quality of either class.

THE TEXTURE OF GRAIN of hexaploid wheat is either hard or soft, each resulting in flour with different processing and end-use characteristics, which depend on protein hydration and development through mixing. Hard wheat is generally used for making bread-type products, and soft wheat is generally preferred for pastry-type products. Hard grain requires more energy to be reduced to flour than soft grain, and its starch granules are damaged more during milling. Damaged starch granules absorb more water, thereby altering several baking properties (Mok and Dick, 1991).

Hybridizations between hard and soft wheat types could expand the genetic base of wheat breeding and create new possibilities for combinations of desirable alleles from both germplasm subgroups. However, this type of cross is not common practice in wheat breeding because the two classes have distinct quality goals. Carver (1996) compared interclass hybrids, backcrosses and progeny from a hard × hard cross, and concluded that the interclass crosses resulted in progenies with higher grain yield but lower flour yield and larger variability for quality traits, and that recovering the quality profile of the hard type through intensive selection would be feasible. Identification of quantitative trait loci (QTL) related to quality differences between classes could help in planning complementary crosses and backcrosses, and in designing selection schemes to recover the quality characteristics needed in either class.

Major genes controlling the difference in kernel texture between soft and hard wheat were mapped on the short arm of chromosome 5D (Sourdille et al., 1996), and as a group are named *Ha* or *Hardness* locus (Baker, 1977). The product of that locus is called friabilin, which is a composite of related proteins that include puroindoline a and puroindoline b (Giroux and Morris, 1998). Other QTLs, with minor effects on kernel texture within the hard wheat type, were detected on chromosomes 1A and 6D (Perretant et al., 2000).

Populations derived from crosses between hard and soft genotypes have higher expected marker polymorphism because of the divergence between the two breeding groups, which can facilitate building a linkage map, compared with other elite × elite crosses. Additionally,

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Abbreviations: AWRC, Alkaline water retention capacity; CIM, Composite interval mapping; SMR, Single marker regression analysis.

there is potential to discover QTLs that are fixed for different alleles within each texture class, which could not be detected in a hard × hard or soft × soft cross. A population derived from the cross of the soft elite line NY18 and the hard variety Clark's Cream has been used to map QTLs of grain quality traits by Campbell et al. (2001). These authors found protein QTLs on groups 1, 2, and 7, and a flour yield QTL on group 3. Markers related to high molecular weight glutenins had major influence on mixogram traits.

In the study of Campbell et al. (2001), the RFLP marker *PinB*, linked to *Ha-5DS*, was highly significantly associated with flour yield, softness equivalent, damaged starch, AWRC, and cookie diameter. However, the effect of the *Hardness* gene was disproportionately large compared with the other loci influencing grain quality, resulting in bimodal distribution of traits affected by that gene. Many statistical methods used in QTL analysis assume normal distribution (Lynch and Walsh, 1998). One approach to meet this assumption when analyzing quantitative variation mixed with qualitative variation is to regress the quantitative trait of interest on the categorical trait and use residuals as phenotypes in the analysis. That approach has been used in genetic analysis of humans and animals to account for effects of sex or race (Grosz and MacNeil, 2001; Friedlander et al., 2003). The effect of a major gene segregating in a mapping population is analogous to those cases and could be controlled by using similar means, provided that categorical variation can be clearly identified.

This paper reports the results of a QTL analysis of several milling and baking quality traits, conducted with the objective of identifying genomic regions related to the variation in kernel quality in a population derived from a cross between a hard and a soft wheat variety. Our focus was on loci other than the known *Hardness* locus; hence, the effect of the segregation of this locus on quality traits was quantified and controlled by regression analysis.

MATERIALS AND METHODS

Plant Materials

The wheat population used in this study consisted of 101 doubled-haploid (DH) lines derived from a cross between 'AC Reed' (Sadasivaiah et al., 1993), a soft white spring wheat cultivar released in 1991 by Agriculture and Agri-Food Canada, Lethbridge Research Centre, and 'Grandin' (PI 531005), a hard red spring wheat cultivar released in 1989 by the North Dakota Agricultural Experiment Station. AC Reed has low protein content and commercially acceptable pastry-type end-use quality. Grandin has high protein content and average bread-type end-use quality. Both cultivars have above average agronomic performance. DH lines were developed at Agriculture and Agri-Food Canada at Winnipeg, MB, Canada. The population was grown in the spring of 1998 at two Canadian locations: Lethbridge, AB, and Swift Current, SK, and at Tulelake, CA, USA. The two Canadian locations have similar climates, with mild summer temperatures (10–25°C) and monthly precipitation between 30 and 70 mm. Tulelake is a warmer (15–30°C) and normally drier environment, although the spring of 1998 offered good growing conditions for wheat (average precipitation of 50 mm/mo). In all locations, the crop was grown under favorable crop conditions.

Genotypic Data and Linkage Map Construction

The genetic map was constructed with 340 molecular markers, including 222 AFLP, 42 RFLP, 75 SSR, and one STS (Lox, Hessler et al., 2002). AFLP markers were evaluated at Agriculture Canada by a modified method based on Vos et al. (1995). All the other markers were evaluated at Cornell University. Hybridization probes included 22 CDO (oat cDNA) and 9 BCD (barley cDNA, Heun et al., 1991), 7 RZ (rice cDNA, Causse et al., 1994), and markers of known genes: PinA (puroindoline a, Giroux and Morris, 1998), MTA9 (esterase, Jouve and Diaz, 1990), and A1tgh (dihydroflavanol reductase, R.A. Graybosch, pers. comm.). SSR markers included 31 WMC (Gupta et al., 2002), 30 GWM (Röder et al., 1998), and 14 BARC (P. Cregan, Q. Song and coll., unpublished). PCR reactions were modified from Röder et al. (1998), and PCR runs consisted of 5 min at 94°C, 35 cycles of 45 s at 94°C, 45 s at specific marker annealing temperature (www.graingenes.org; verified 15 April 2005), and 90 s at 72°C, followed by a 10 min final extension at 72°C.

The linkage map was constructed with Map Manager QTX13 (Manly and Olson, 1999) using the Kosambi mapping function and type-I error probability of 0.001, followed by "ripple" command to check the ordering of markers within each linkage group. Genetic markers presenting high segregation distortion by the χ^2 test ($P < 0.001$) were not used in the first stage of map construction, when the core map was defined. In a second round, those markers were allowed to link to the existing groups using the "distribute" command, so that they could be used for QTL analysis by composite interval mapping. The resulting linkage groups were compared to the Synthetic W7984 × Opata 85 map (Marino et al., 1996; Nelson et al., 1995; Van Deynze et al., 1995) and tentatively assigned to wheat chromosomes on the basis of common markers. Mapchart (Voorips, 2002) was used to draw the linkage map with QTLs.

Phenotypic Evaluations

Seed was thoroughly air-aspirated to remove shriveled kernels and nonwheat materials before evaluation for milling and baking quality parameters at the USDA-ARS, Soft Wheat Quality Laboratory, at Wooster, OH. Four traits were evaluated on samples from three locations: Quadrumat mill flour yield and softness equivalent, according to Finney and Andrews (1986), flour protein content and alkaline water retention capacity (AWRC), following AACC Approved Methods 46-12 and 56-10, respectively (www.aaccnet.org; verified 15 April 2005). A sample of 500 g of grain from Tulelake was Allis-Chalmers milled, yielding Allis-Chalmers flour yield, break-flour yield, endosperm separation index (Yamazaki and Andrews, 1977), and friability. Friability was the ratio of the weight of flour produced divided by the sum of the weight of the mill stock fed to each of the break and reduction rolls. The consistency of the Allis-Chalmers milling data were evaluated by analysis of variance of two independent samples from each of seven checks [AC Reed, Grandin, 'Serra' (CI 87738), 'Yolo' (CI 17961), 'Katepwa' (AFRC 5621), 'Anza' (CI 15284), and 'UC896' (University of California-Davis experimental line)]. The check samples were produced in the same year and location as the mapping population. Ten-gram mixograph assay (Finney and Shogren, 1972) resulted in mixing time and peak height, plus mixogram curve area and area under the curve determined by image analysis of the mixogram. Addi-

tional tests included: kernel volume (AACC Method 55-10), sucrose retention capacity and lactic acid retention capacity (AACC Method 56-11), cookie diameter, and top grain (AACC Method 10-52).

QTL Analysis

The DH lines were classified as hard or soft type on the basis of the mean softness equivalent score (Yamazaki and Andrews, 1977). Phenotypic data were regressed on this classification, and residual variance was used for QTL analysis. The purpose of this adjustment was to eliminate the qualitative-like variation caused by segregation of the *Ha-5DS* locus (Sourdille et al., 1996), and achieve normal distribution of data, as checked by Shapiro-Wilk test at $\alpha = 0.01$ (SAS System V.8). QTL analysis was performed in QTL Cartographer V2.0 (Wang et al., 2001). All traits were analyzed by single marker regression analysis (SMR) using all markers.

Replicated traits (Quadrumat flour yield, softness equivalent, protein content, and AWRC) were analyzed by composite interval mapping (Zeng, 1994), using a reduced version of the map, including only linkage groups containing at least one locus significant for any trait at $\alpha = 0.01$ in the previous SMR analysis. The parameter settings for CIM were model 6, stepwise selection of cofactors with SLE = SLS = 0.01, window size 10 cM and testing step 2 cM. Experiment-wise type I error rate of detected QTLs was estimated by one thousand permutations with the same settings (Churchill and Doerge, 1994).

Multiple-trait analysis method was used to jointly analyze QTL over locations using each location as a "correlated trait" (Jiang and Zeng, 1995). A threshold of LOD = 3 was arbitrarily applied for those QTLs. Approximate 95% confidence intervals were built by concatenating adjacent positions within a 1-LOD difference from the value of the peak, although those intervals tend to have an actual probability level lower than 95% (Mangin et al., 1994), and the interval probability is unknown when it is truncated by the end of the linkage group.

Analysis of variance of phenotypes for locations and lines was done, and significance was calculated using the variance of location × line interaction as error. Multiple regression models were built, beginning with markers within the confidence interval of joint analysis plus the significant markers by SMR at $\alpha = 0.01$. Predictors were selected by backward elimination with SLS = 0.05 for main effects and SLS = 0.01 for interaction effects (SAS System V.8).

RESULTS

Genetic Map

The genetic map of the AC Reed × Grandin population (Fig. 1) consisted of 43 linkage groups, including 320 loci, and 20 markers remained unlinked. The total map length was 3555 cM and the mean marker-interval was 12.8 ± 9.0 cM (min = 2.2; max = 42.1 cM). Forty of the linkage groups could be tentatively assigned to wheat chromosomes through common markers in the W7984 × Opata 85 map. Twelve linkage groups were assigned to genome A, 16 to genome B, 7 to genome D, 3 were unresolved between genomes A and B, and 2 were unresolved between genomes A and D. Forty-eight loci (47 AFLPs and the microsatellite locus *Xwmc44*) had highly significant segregation distortion (χ^2 $p < 0.001$). Thirty-seven loci were skewed toward AC Reed, most of them mapped to the linkage group 1AD. The largest skewed region toward Grandin was on 1BL-2, including 6 consecutive markers.

Phenotypic Traits

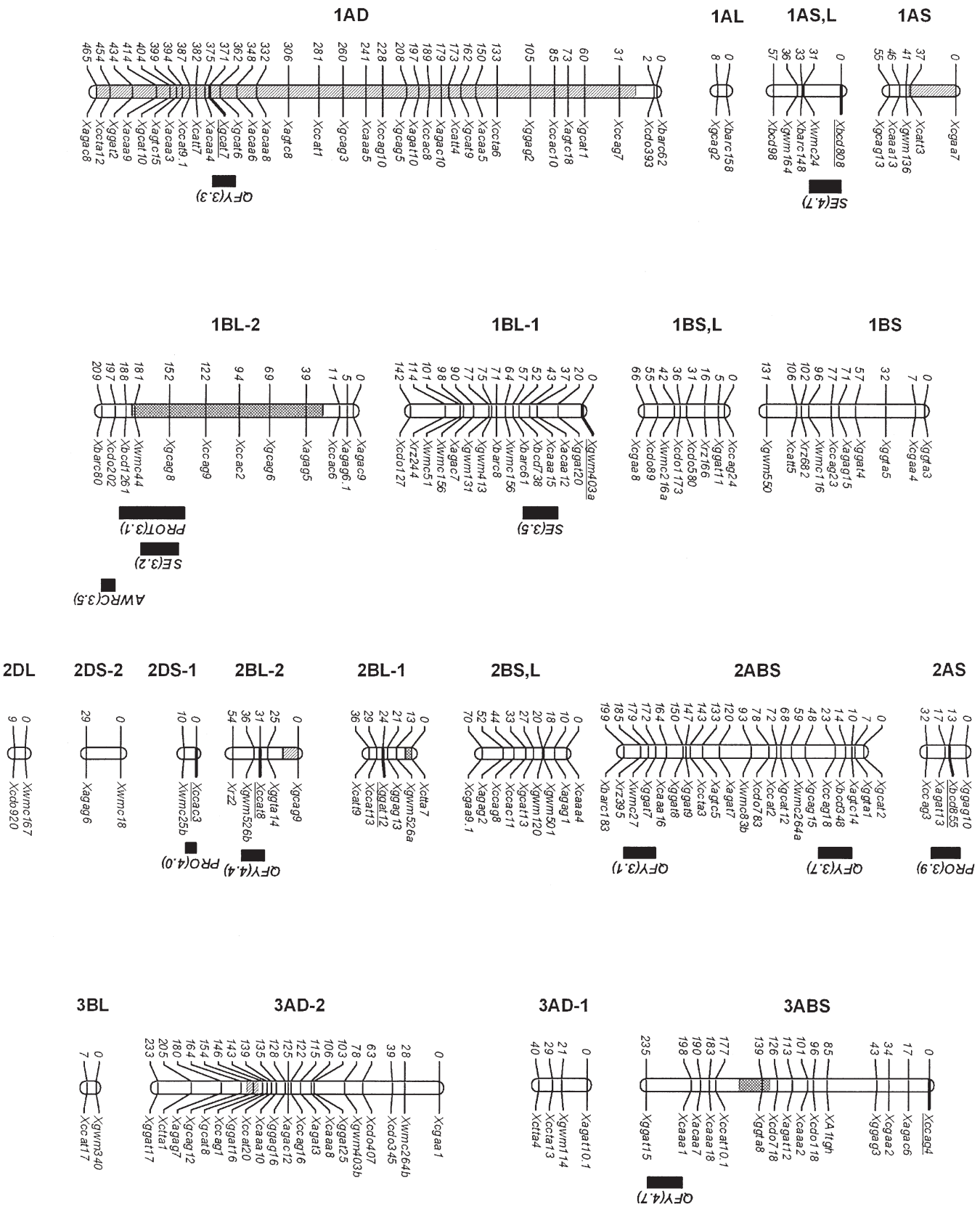
The DH lines segregated into two phenotypic classes of kernel texture. Forty-five lines were hard, with low softness equivalent scores, and 56 lines were soft, with high softness equivalent scores ("original" data, Fig. 2). The residual variance after removing the effect of texture class fitted the normal distribution well ("adjusted" data, Fig. 2). Texture class assignment was totally consistent across locations, and had a large effect for the majority of the milling and baking traits (Table 1). That classification accounted for approximately 90% of the variance for softness equivalent and break flour yield, and approximately half of the variance for Quadrumat flour yield, Allis-Chalmers flour yield and friability. The effects on protein content were moderate and different among locations, with a maximum of approximately 15% of the variance at Lethbridge, but a nonsignificant effect in Tulelake. Texture class did not affect kernel volume.

Quantitative Trait Loci

In this study, we considered QTLs as genomic positions other than *Ha-5DS* with significant effects on quality traits. The r^2 values of the QTLs refer to the residual variance after adjustment of phenotypes for texture classes, hence are comparable to values found in previous studies using intraclass crosses. Single-marker regression analysis (SMR) identified 65 markers highly significant ($P < 0.001$) for at least one of the traits (Fig. 3). Most markers were significant for more than one trait. Composite interval mapping (CIM) detected 15 QTL effects, on 13 linkage groups, putatively related to wheat chromosomes 1A, 1B, 1A/D, 2A, 2B, 2D, 3A/B, 4B, 5B, 6B (Table 2). Nine of the QTL effects were detected on genome B. Coefficients of determination ranged from 9.9 to 40.3%, with an average of 16.7%.

Quadrumat Flour Yield

Significance of markers by SMR was reasonably consistent for Quadrumat flour yield across environments (Fig. 3). CIM identified QTLs in Tulelake on 2DS-1, 3ABS, 4B-2, and 5BL, and at Lethbridge on 2BL-2 (Table 2). No QTL was detected by CIM for this trait at Swift Current. AC Reed, the low flour yield parent, contributed alleles with increasing effect on 4B-2 (Tulelake) and 2BL-2 (Lethbridge). Genomic regions on linkage groups 1AD, 2ABS, 2BL2, 3ABS, and 4B-2 were significantly related to Quadrumat FY by joint analysis of the three environments (Fig. 1). One of those regions, on 4B-2, coincides with a QTL for AWRC. In ANOVA, locations had a large effect for Quadrumat FY (r^2 = 0.801, but the interaction location × line was very small, indicating consistent ranking of genotypes. The multiple regression model for Quadrumat FY included seven markers and one interaction, and explained approxi-



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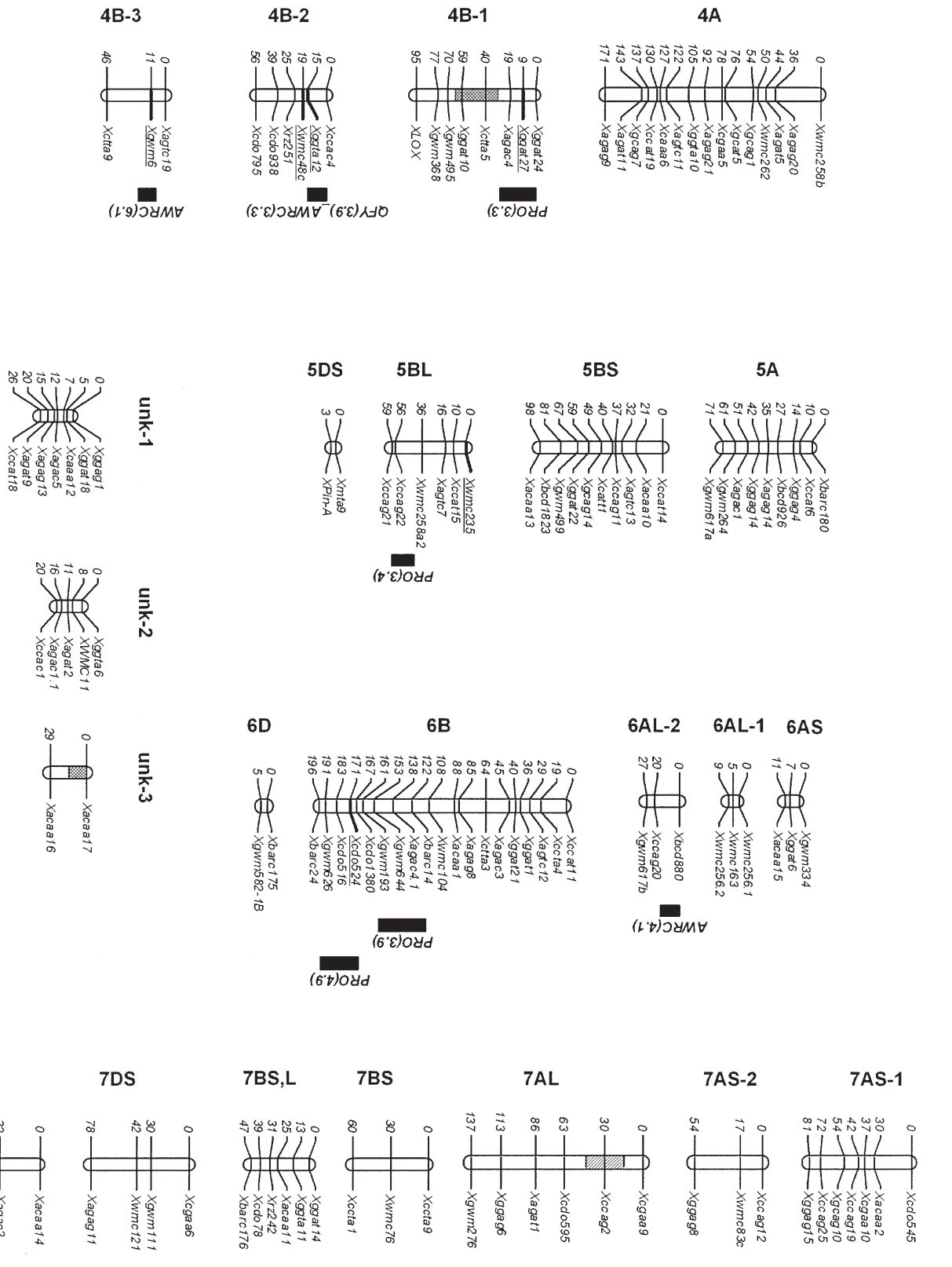

Fig. 1. Genetic linkage map of the AC Reed × Grandin wheat population, based on recombination among 101 DH lines. Linkage groups are named by homoeologous group, genome and chromosome arm. Three groups were not identified (Unk1-3). Diagonal lines or criss-cross pattern on the chromosome bars indicate segregation distortion (χ^2 $p < 0.001$) toward AC Reed or Grandin, respectively. Underlined font indicates loci close to QTL peaks detected by CIM (details in Table 2). Solid bars indicate approximate 95% confidence intervals for QTLs detected by joint-analysis of locations, with peak LOD scores in parenthesis. QFY: Quadrumat flour yield; SE: softness equivalent; PRO: flour protein content; AWRC: alkaline water retention capacity.

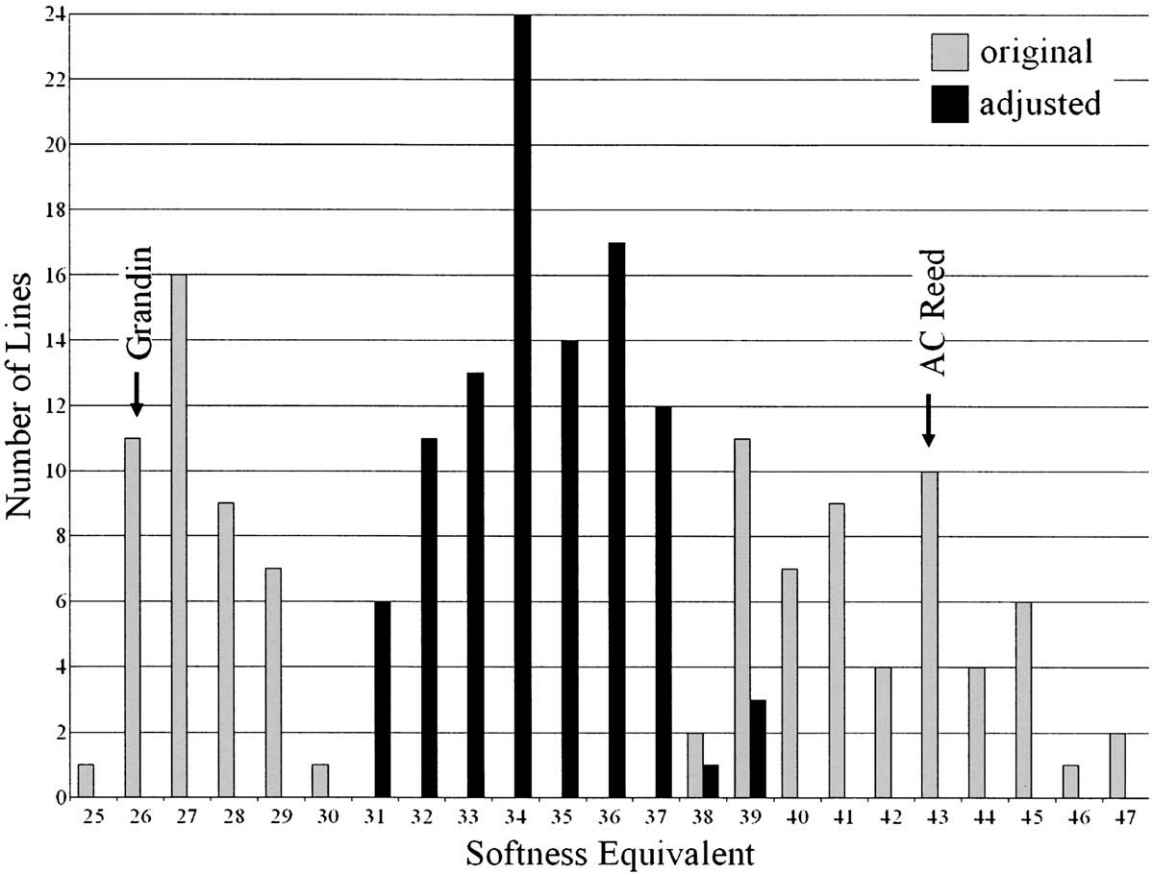

Fig. 2. Distribution of average of softness equivalent over three locations before and after adjustment for texture class (original and adjusted, respectively). Forty-five lines had low original SE and were classified as hard; 56 lines had high original SE and were classified as soft. The phenotypes of the parents are indicated by arrows.

mately 60% of the phenotypic variance in all locations (Table 3).

Softness Equivalent

SMR analysis identified many loci significantly associated with softness equivalent (Fig. 3), and CIM mapped QTLs on 1AS,L, 1BL-1, and 1AD (Table 2). Grandin, the hard parent, contributed the allele for increased softness at the QTL on 1AS,L. Joint analysis confirmed the QTLs on 1AS,L and 1BL-1, plus a region on 1BL-2 (Fig. 1). The main effect of locations in the ANOVA was large (r^2 = 0.743), but the interaction of line × location was relatively small (r^2 = 0.059). The multiple regression model included 2 markers and one interaction effect, and explained proportions of the phenotypic variance ranging from 17.2% at Swift Current, to 34.8% at Lethbridge (Table 3).

Protein Content

Three QTLs for protein content were detected by CIM at Tulelake, on linkage groups 2AS, 4B-1 and 6B, and two QTLs were detected in Swift Current, on 2BL-1 and 4B-2 (Table 2). All of those regions were significant by SMR (Fig. 3). No QTL was found for Lethbridge by CIM, and the single markers were only marginally significant. Joint analysis detected QTLs on linkage groups 1BL-2, 2AS, 2DS-2, 4B-1, 5BL and in 6B, which was the most significant (LOD = 4.9, Fig. 1). The main effect of location in the ANOVA of protein content was very small (r^2 = 0.022), but the location × line interaction was the highest among the traits studied (r^2 = 0.296). A multiple regression model included seven markers but no interaction effects. Together, those markers explained proportions of the variance varying from 36.5% at Lethbridge to 65.6% at Tulelake (Table 3).

Alkaline Water Retention Capacity

AWRC was correlated with markers on 1BL-2 and 4B-3, plus other minor effects (Fig. 3). CIM detected the QTL on 4B-3 for Swift Current, which had the highest coefficient of determination in our study (r^2 = 0.403, Table 2). AC Reed, the lower parent for AWRC, contributed the increasing allele at that QTL. Joint analysis of environments detected the QTL on 4B-3 with LOD = 6.1, plus three other significant regions on 1BL-2, 4B-2, and 6AL-2 (Fig. 1). In the ANOVA, main effect of location and the location × line interaction on AWRC was intermediate compared with the other traits. A regression model composed of three markers explained a modest proportion of the phenotypic variance (Table 3).

Table 1. Summary statistics of grain quality traits in the AC Reed × Grandin wheat population. Phenotypes of the parents, coefficient of determination of texture classes, and descriptive statistics of adjusted data†. LB: Lethbridge; SC: Swift Current; TL: Tulelake. All other traits evaluated in Tulelake.

			Parents			Adjusted data		
Trait	Locations	Unit	AC Reed (soft)	Grandin (hard)	Texture class r^2	Mean	SD	Range
Quadrumat flour yield	LB	%	72.6	74.0	0.443**	73.15	0.91	70.3–74.9
	SC	%	72.3	73.9	0.433**	72.96	0.88	69.9–74.6
	TL	%	76.0	78.4	0.618**	77.09	0.96	74.2–78.9
Softness equivalent	LB	%	46.6	28.3	0.902**	37.03	2.35	32.0–43.2
	SC	%	45.4	28.2	0.939**	36.00	1.98	32.1–41.5
	TL	%	35.5	21.2	0.922**	28.96	1.99	25.1–34.5
Flour protein content	LB	%	8.9	10.6	0.148**	10.08	0.52	8.98–11.37
	SC	%	9.4	11.2	0.100*	10.29	0.57	8.94–11.62
	TL	%	8.7	11.2	0.040	10.13	0.69	8.57–11.45
Alkaline water retention capacity	LB	%	55.5	63.5	0.790**	59.54	1.44	54.6–65.7
	SC	%	55.0	63.5	0.856**	59.38	1.17	56.3–61.5
	TL	%	52.5	63.4	0.934**	57.55	1.22	55.0–61.1
Allis-Chalmers flour yield		%	76.7	78.4	0.523**	76.90	0.82	74.6–78.6
Break flour yield		%	23.5	14.6	0.894**	18.47	1.24	16.0–22.7
Friability		%	26.1	24.9	0.535**	25.32	0.88	23.1–27.7
Endosperm separation index		%	8.9	7.8	0.259**	9.36	0.97	7.5–11.6
Kernel volume		#/bu	64.3	64.6	0.037	64.78	0.79	63.2–66.4
Mixogram area under curve		cm^2	4114	5038	0.039*	4601	461	3577–5679
Mixogram curve area		cm^2	19.1	38.0	0.020	32.57	6.44	18.6–47.7
Mixogram height		cm	3.80	4.87	0.010	4.42	0.35	3.43–5.29
Mixogram time		min	1.70	2.95	0.191**	2.86	0.90	1.36–5.06
Lactic acid retention capacity		%	68.7	109.1	0.051*	97.23	9.36	70.2–114.7
Sucrose retention capacity		%	87.1	97.6	0.641**	92.93	2.40	88.2–99.7
Cookie diameter		cm	18.3	15.6	0.891**	16.76	0.30	16.0–17.4
Top grain		score	7.50	3.50	0.675**	4.97	1.05	1.49–7.01

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
† Phenotypes were adjusted by linear regression on texture class (hard or soft) as defined by softness equivalent scores.

Allis-Chalmers Milling Traits

The Allis-Chalmers milling data for the population were unreplicated. An approximate error associated with those determinations was derived from two replicates of each of seven checks, grown in the same field and year. The error variance of all of the Allis-Chalmers traits was very small (Table 4), with cultivars accounting for more than 95% of the variation for all traits except kernel volume. The standard deviations among lines for the same traits (Table 3) were two- to four-fold greater than the error among checks, implying that a large proportion of the phenotypic variance was due to genetic differences among lines. The locus *Xgcat6* on 1AD was highly significant ($P < 0.001$) for Allis-Chalmers milling flour yield and friability and marginally significant ($P < 0.05$) for break flour yield and endosperm separation index (Fig. 3). AC Reed had the favorable allele at that locus, increasing flour yield and softness. Another region influencing friability was found on 1BL-2, with increasing effect from Grandin. A QTL for break flour yield was detected by *Xbcd808* on 1AS,L, in a region apparently involved with softness. Three highly significant markers for kernel volume were found on 2ABS, 2BS,L, and 4B-3. Grandin contributed the increasing alleles for those three loci (Fig. 3).

Mixogram Traits

All four mixogram traits were correlated with loci on linkage groups 1BS,L and 1BL-1 (Fig. 3). The locus *Xwmc216a-1BS,L* was the most influential, explaining 29.4% of the variance of mixing time (results not shown). Alleles from AC Reed reduced mixing time and curve area and increased mixogram height and area under curve on both 1BS,L and 1BL-1. Markers on 3ABS correlated with mixogram height, area under curve, and time, but in this case, the direction of the effects was reversed. Mixogram height was influenced by other markers located on 2AS and 3AD-2 (Fig. 3).

Baking Assay Traits

Lactic acid retention capacity correlated positively with AC Reed alleles on 1AS (Fig. 3). A region affecting sucrose retention capacity was detected on 4B-2, with the increasing effect from Grandin. Cookie diameter was influenced by a QTL on 6B, with positive effect from Grandin. Top grain was related to markers on 2ABS and 4B-3, both with favorable effect from AC Reed.

DISCUSSION

The AC Reed × Grandin DH population presented in this paper, coupled with a genetic map with reasonable genome coverage, was useful for exploratory QTL mapping of quality traits. Segregation distortion was observed in some parts of the map. This phenomenon is common in double-haploid populations and seems to be related to loci affecting viability in anther culture (Manninen, 2000). However, Hackett and Broadfoot (2003) studied the effects of segregation distortion on linkage mapping and found minimal effects on marker order or map length. We used the ad hoc procedure of building the framework map with nonskewed markers and then distributing skewed markers over the existing linkage groups, as a mean to reduce a possible impact of distortion in the map.

The D-genome was populated with fewer markers as

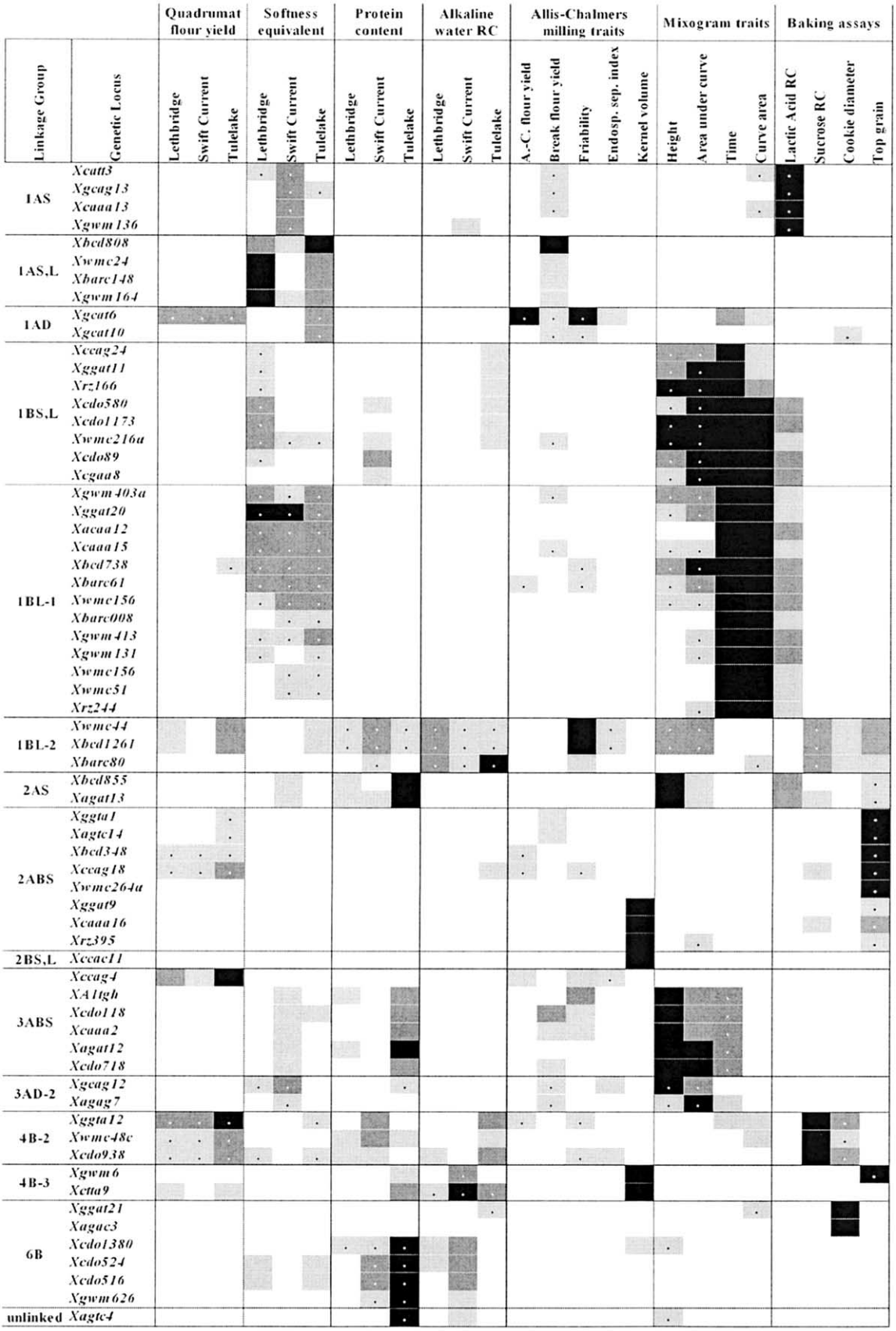

Fig. 3. Summary of results of single marker regression analysis of grain quality traits in the AC Reed × Grandin DH population. Light gray, dark gray, and black indicate significance at the 0.05, 0.01, and 0.001 level, respectively. Only markers that were significant at 0.001 level for at least one trait are shown. A dot indicates increasing effect from AC Reed. Markers are in mapping order within linkage groups. Allis-Chalmers milling, mixogram, and baking traits were evaluated at Tulelake only.

Table 2. Quantitative trait loci detected in the AC Reed × Grandin wheat population by composite interval mapping. Additive effect direction is for the allele from AC Reed. Loc: locations; LB: Lethbridge; SC: Swift Current; TL: Tulelake.

Linkage group	Trait	Loc.	Peak position† (cM)	Closest locus	r2	Additive effect	LOD score	Prob.‡
1AS,L	softness equivalent	TL	6.0	*Xbcd808*	0.155	−0.78	3.81	0.008
1AS,L	softness equivalent	LB	16.0	*Xbcd808*	0.192	−1.03	3.84	0.015
1AD	softness equivalent	TL	371.4	*Xgcat7*	0.119	0.90	3.10	0.048
1BL-1	softness equivalent	SC	0.0	*Xgwm403a*	0.124	0.70	3.34	0.041
2AS	flour protein content	TL	15.5	*Xbcd855*	0.213	−0.32	7.22	<0.001
2BL-1	flour protein content	SC	24.4	*Xggat12*	0.143	0.22	4.34	0.005
2BL-2	Quadrumat flour yield	LB	33.2	*Xccat8*	0.107	0.31	3.45	0.033
2DS-1	Quadrumat flour yield	TL	0.0	*Xccac3*	0.116	−0.34	3.75	0.010
3ABS	Quadrumat flour yield	TL	4.0	*Xccag4*	0.219	−0.45	5.41	<0.001
4B-1	flour protein content	TL	13.3	*Xggat27*	0.099	0.22	3.64	0.031
4B-2	Quadrumat flour yield	TL	10.0	*Xggta12*	0.193	0.43	5.35	<0.001
4B-2	flour protein content	SC	18.7	*Xwmc48c*	0.123	−0.20	3.79	0.016
4B-3	alkaline water retention capacity	SC	12.0	*Xgwm6*	0.403	0.75	6.46	<0.001
5BL	Quadrumat flour yield	TL	0.0	*Xwmc235*	0.121	−0.35	4.01	0.004
6B	flour protein content	TL	169.0	*Xcdo524*	0.184	0.30	6.49	<0.001

† Testing position with the highest LOD score in the region.
‡ Experiment-wise type I error rate based on one thousand permutations.

a consequence of lower polymorphism compared with A and B genomes, as observed in previous studies (e.g., Cadalen et al., 1997; Marino et al., 1996). More complete linkage maps normally require populations derived from wide crosses; however, this type of population frequently presents limitations to QTL analysis because of lack of agronomic adaptation. Adapted genotypes and their progeny are more likely to express their genetic potential under normal growing conditions and to produce fully developed grain for quality assessment. In this study, both parents were highly adapted to the growing conditions of the tested locations.

Hard and soft wheat varieties are normally developed by separate breeding programs; hence, the polymorphism of the AC Reed × Grandin cross was probably higher than that of a cross within texture class. However, the segregation of the hardness locus *Ha-5DS* introduced a "nuisance" variance in the population. This was a similar situation to human or animal populations where the effect of sex or race can override the expression of genes of interest (Grosz and MacNeil, 2001; Friedlander et al., 2003). Removing the effect of *Ha* by regression facilitated the detection of minor QTLs.

Single marker regression is the simplest method of QTL analysis. Although this method has some disadvantages, such as low power and biased estimation of QTL effects (Lander and Botstein, 1989), its results allowed a thorough comparison of locus-wise significance across traits. QTLs detected through CIM above thresholds that are corrected for multiple testing normally do not allow such straightforward comparison because many moderate but nonetheless real effects are not detected. A conservative threshold may result in different QTLs being detected in each location and could overestimate the importance of the QTL × environment interaction. The use of lower thresholds would probably result in better agreement among different environments and studies, but in this case, the protection of the experiment-wise type I error rate would require larger population sizes. On the other hand, CIM allows for a more precise location of important QTLs within linkage groups and better estimation of the QTL effects (Zeng,

Table 3. Summary of analysis of variance of four grain quality traits of 101 DH lines evaluated at three locations and multiple regression analysis on genotypes at selected loci, by location. Values are the proportion of the total sum-of-squares explained by each factor in ANOVA or the model r^2 in the regression analysis.

Statistical analysis	Quadrumat flour yield	Softness equivalent	Flour protein content	Alkaline water retention capacity
ANOVA				
Locations	0.801***	0.743***	0.022**	0.366***
Lines	0.186***	0.198***	0.682***	0.429***
Location × line (residual)	0.013	0.059	0.296	0.204
Multiple regression				
Genetic loci in the model	*Xccac3*	*Xcaaa15*	*Xccag22*	*Xctta9*
	Xbcd348	*Xwmc24*	*Xcdo202*	*Xcdo202*
	Xccac4		*Xagat13*	*Xggta12*
	Xwmc24		*Xccac3*	
	Xccag4		*Xggat27*	
	Xccat8		*Xggta12*	
	Xggat25		*Xcdo1380*	
Locus interactions	*Xccat8* × *Xctta9*	*Xccac3* × *Xccac4*	–	–
Number of lines used†	75	76	72	82
Lethbridge	0.630***	0.348***	0.365***	0.148**
Swift Current	0.634***	0.172*	0.415***	0.237***
Tulelake	0.595***	0.285***	0.656***	0.205***

* Significant at the 0.05 probability level.
** Significant at the 0.001 probability level.
*** Significant at the 0.0001 probability level.
† Because of missing marker data, part of the lines were excluded from multiple regression analysis.

Table 4. Results of analysis of variance of Allis-Chalmers milling traits from seven checks with two replicates each in completely randomized design.

ANOVA results	Kernel volume	Allis-Chalmers flour yield	Endosperm separation index	Friability	Break flour yield
r^2 of checks	0.869	0.951	0.986	0.973	0.994
Trait mean	64.63	78.15	7.957	25.87	17.97
Sqrt (mean square error)	0.210	0.393	0.264	0.353	0.296
Coefficient of variation	0.325	0.503	3.318	1.365	1.647

1994). Therefore, the results of the two methods complemented each other in this study.

Rapid microtests for milling quality that identify the same genes as commercial scale milling evaluations are critical for wheat millers and breeders. Previous reports described a modified Quadrumat mill that produced flour yield data that ranked cultivars similarly to the longer-flow Allis-Chalmers mill (Finney and Andrews, 1986) but noted that grain moisture content and texture strongly influenced the results. Gaines et al. (2000) developed algorithms to adjust Quadrumat flour yield to 150 g kg^{-1} moisture and a constant softness equivalent based on particle size, which raised the correlation between the results from the two mills from 55 to 90%, without having to temper the wheat before Quadrumat milling. However, phenotypic correlations often do not accurately reflect a common genetic basis for two traits. In this study, we compared the QTL for Quadrumat flour yield with the Allis-Chalmers flour yield (Fig. 3). All markers that were significant for Quadrumat flour yield were also significant for either Allis-Chalmers flour yield or friability or both and Allis-Chalmers flour yield identified only one QTL that was not detected by the Quadrumat (*Xbarc61-1BL-1*). These results validate the use of the modified Quadrumat mill and the algorithms of Gaines et al. (2000) for predicting flour yield and friability of Allis-Chalmers mill for selecting wheat genotypes with superior milling characteristics.

Some of the QTLs reported here may have a common genetic base with previously reported QTLs. The locus *Xbcd1431* on 4DL-2 was related to AWRC and damaged starch in the study of Campbell et al. (2001) and may be homoeologous to the QTL for AWRC (Swift Current) on 4B-3 in this study. Our QTL for kernel volume on 2ABS agreed with a major QTL reported by Campbell et al. (1999). The largest QTL for Quadrumat flour yield on 3ABS may be the same reported on 3A in bread wheat by Parker et al. (1999). QTLs for softness equivalent were detected here on linkage group 1AS,L, which may match a QTL for kernel hardness previously reported on 1A (Perretant et al., 2000).

The highly significant QTL for protein content that we found on 2AS could refer to the same gene as one reported on chromosome 2A of other bread wheat populations by Groos et al. (2003) and Prasad et al. (2003). A QTL for protein content was mapped close to *Xgwm193-6B* by Khan et al. (2000). In our map, that marker is between two non-overlapping confidence intervals of protein QTLs on 6B. However, a more conservative criterion (e.g., 2-LOD drop) would merge the two confidence intervals (result not shown), hence the possibility of a single QTL cannot be eliminated. We found QTLs for protein content on 4B-1 (Tulelake) and 4B-2 (Swift Current) and confirmed by joint analysis on 4B-1, which could match the QTL on 4B reported to be the most stable across environments by Blanco et al. (2002).

Some mixogram QTLs agreed with previous QTLs or known genes. On the basis of the linked loci *Xrz166* and *Xcdo1173*, we infer that the effect detected by *Xwmc216a-1BS,L* was probably caused by the gliadin *Gli-B3* gene (Galili and Feldman, 1984). The gene underlying the QTL for mixogram time and curve area near *Xbcd738-1BL-1* was probably the glutenin *Glu-B1* gene (Payne et al., 1982), which is near the locus *Xbarc61* (5 cM from *Xbcd738*) in the W7984 × Opata 85 map. The locus *Xagat12*, near the QTL for mixogram height on 3ABS, was closely linked to *Xcdo718*, which was related to flour viscosity in the population NY18 × Clark's Cream (Udall et al., 1999).

Several QTLs had effects opposite to the phenotypes of the parents. This was expected because parental phenotypes were largely defined by their alleles for *Ha-5DS*. Grandin, the hard wheat parent, contributed the allele for softness at a QTL on 1AS,L (Table 2) and for larger cookie diameter on 6B. Similarly, the Grandin allele at the QTL near *Xwmc44-1BL-2* was favorable for soft wheat products, increasing friability, flour yield, cookie diameter, and top grain, while decreasing protein content, AWRC, sucrose retention capacity, and mixogram height (Fig. 3). On the other hand, AC Reed alleles increased protein content at the QTLs on 2BL-1, 4B-1, and 6B, and flour yield at QTLs on 2BL-2 and 4B-2 (Table 2). Those sources of variation represent opportunities to improve quality traits through hard × soft hybridization. QTLs reported here and in previous papers could orient marker-assisted selection strategies to accelerating the recovery of quality characteristics required for each class following hybridizations while retaining favorable alleles from both parents.

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